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SphaeraMag[®] Genomic DNA Tissue and Hair Purification Kit

Nucleic Acid Extraction
for Manual and Automated Systems

Laboratory Use Only

Instruction Manual
Version 1.7

LICENSE AGREEMENT FOR THE MANUAL








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Explanation of Symbols

	Catalogue Number		Batch Code
	Consult instructions for use		Manufacturer
	Use by date		Temperature limit
	Contains sufficient for <n> tests		

1. INTRODUCTION

The SphaeraMag® Genomic DNA Tissue and Hair Purification Kit is designed for rapid and reliable isolation of genomic DNA (gDNA) from tissue and hair samples. Samples are lysed in a specifically formulated buffer to release nucleic acids. The gDNA is bound to the surface of paramagnetic beads, while proteins and cellular debris are removed during washing steps. The gDNA is then eluted in the provided Elution Buffer. Alternatively, nuclease-free water or other low ionic strength buffers can be used. The procedure can be performed using automated systems (like Phoenix-Pure) or manually. The purified gDNA is suitable for numerous downstream applications such as qPCR, end-point PCR, SNP analysis, genotyping, microarray analysis and library preparation for NGS sequencing.

2. KIT COMPONENTS

	SphaeraMag® Genomic DNA Tissue and Hair Purification Kit - Universal 96	SphaeraMag® Genomic DNA Tissue and Hair Purification Kit - Pre-filled 96	SphaeraMag® Genomic DNA Tissue and Hair Purification Kit - Pre-filled 32	SphaeraMag® Genomic DNA Tissue and Hair Purification Kit - Pre-filled Mini
Catalogue No.	PCCSKU16088	PCCSKU16089	PCCSKU16090	PCCSKU16091
Preps	96	96	80	48
Lysis Buffer	20 ml	20 ml	20 ml	10 ml
Binding Buffer	20 ml	pre-filled	pre-filled	pre-filled
Wash Buffer I	47 ml	pre-filled	pre-filled	pre-filled
Wash Buffer II	27 ml	pre-filled	pre-filled	pre-filled
Elution Buffer	10 ml	pre-filled	pre-filled	pre-filled
Proteinase K	2 x 1 ml	2 x 1 ml	2 x 1 ml	1 ml
Magnetic Beads	1 ml	1 ml	1 ml	1 ml
Tip comb(s)	-	1 x 96 tip comb (in holder)	10 x 8 tip combs	30 x 8 tip combs

Table 1. SphaeraMag® Genomic DNA Tissue and Hair Purification Kit content.

3. KIT COMPATIBILITY

	Universal 96	Pre-filled 96	Pre-filled 32	Pre-filled Mini
Phoenix-Pure 96/Auto-Pure 96	+	+		
Phoenix-Pure 32/Auto-Pure 32	+		+	
Auto-Pure Mini	+			+
KingFisher™ Flex Purification System	+	+		

Table 2. SphaeraMag® Genomic DNA Tissue and Hair Purification Kit compatibility.

4. STORAGE AND STABILITY

Proteinase K enzyme must be stored at -25 to -15°C, Magnetic Beads at +2 to +8°C upon arrival. All other kit components are stored at room temperature (+15 to +25°C). The expiry dates of the kit and single components are stated on the outer packaging as well as on the individual reagents.

5. PRECAUTION

Proper laboratory safety practices should be employed, including the use of lab coat, gloves and protective goggles. For information regarding the composition of buffers, please check the Safety Data Sheets (available upon request). Each reagent is optimized for the specific use with this kit. Do not substitute reagents from any other manufacturer into the kit and do not combine with different Lot numbers.

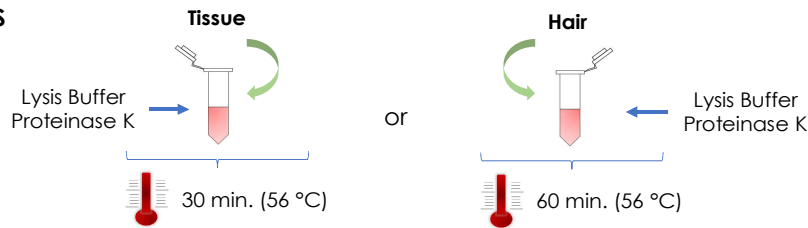
6. PROCEDURE OVERVIEW

Tissue or hair samples are lysed in a chaotropic salt-based Lysis Buffer to release the gDNA. In the following step, a Binding Buffer is added to allow binding of the gDNA to the surface of paramagnetic beads. A three step washing procedure, including two washing buffers, is performed to remove proteins, detergents and other impurities. The gDNA is then released from the Magnetic Beads using Elution Buffer.

To prevent the co-isolation of RNA during the gDNA extraction, an optional RNase A (not supplied) treatment can be included in the protocol.

7. ILLUSTRATED PROTOCOL

PART 1: SAMPLE LYSIS



PART 2: BINDING AND ELUTION

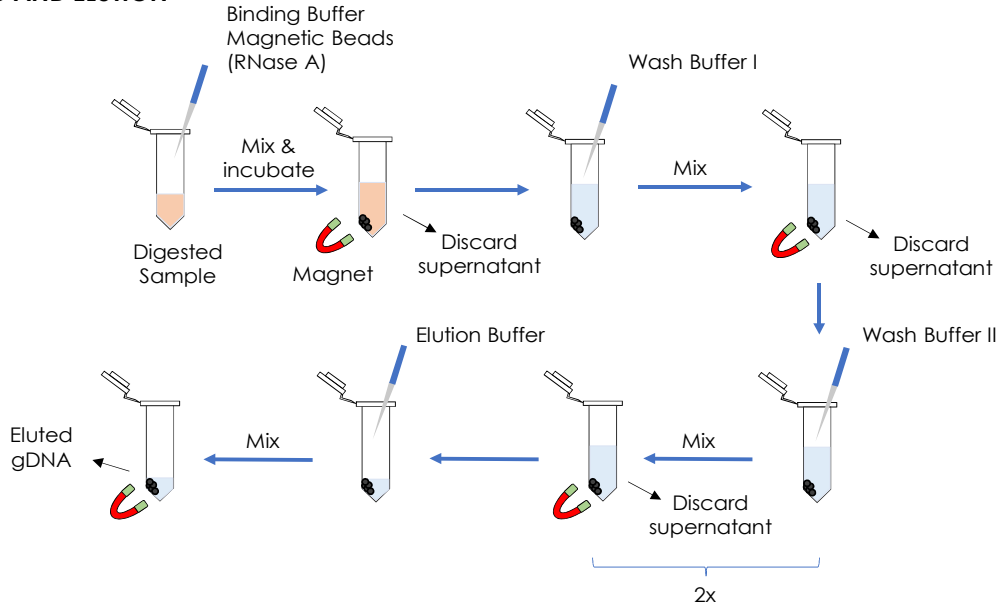


Figure 1. Illustrated protocol for manual gDNA isolation.

8. REQUIRED MATERIALS NOT SUPPLIED

General

- Ethanol, absolute (only for Universal 96)
- Isopropanol $\geq 99.5\%$ (only for Universal 96)
- (Optional) RNase A - recommendation: 20 mg/ml solution
- Suitable reagent and sample dispensing options
- Consumables for isolation devices for automated extraction
- Vortexer
- Thermal mixer for 1.5 ml or 2 ml tubes (or alternative heating device)
- 1.5 ml or 2 ml microcentrifuge tubes (ideally low binding)

Specific for manual purification

- Magnetic separation rack for 1.5 ml or 2 ml tubes
- Water bath (or alternative heating device to pre-heat buffers)

9. SPECIFICATIONS

Recommended sample input	Tissue: 100 mg Hair (incl. bulbs): 20 pcs
Yield	Tissue: 12-40 µg (depending on origin) Hair: 8-14 µg
Genomic DNA size	Peak size >50 kb
Purity	A260/280 >1.8 A260/230 >2.0

Table 3. Kit specifications.

10. PERFORMANCE AND DOWNSTREAM APPLICATIONS

The SphaeraMag® Genomic DNA Tissue and Hair Purification Kit generates excellent input material regarding DNA integrity and purity for downstream applications such as quantitative PCR.

DNA Integrity

The gDNA samples were analyzed regarding DNA integrity by loading 10 µl eluate on an 1% agarose gel. Representative gDNA samples obtained from human hair and bovine lung tissue are shown.

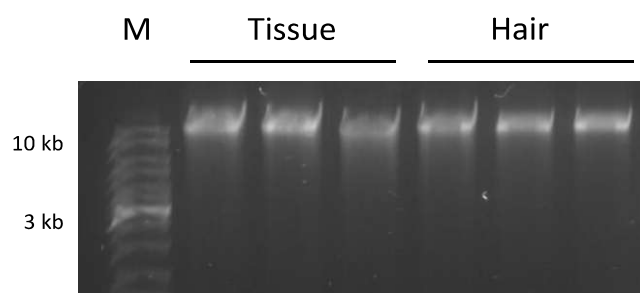


Figure 2. Integrity of gDNA isolated from bovine lung tissue and human hair assessed by agarose gel electrophoresis.

Quantitative PCR

Purified gDNA from representative bovine lung and human hair samples was diluted to produce a four-log range of input template concentrations. Results were obtained using primers targeting bovine- and human-specific genomic sequence for qPCR assays with the PhoenixDx® ABC qPCR Mastermixes (Procomcure Biotech #PCCSKU12011) and cycled on Applied Biosystems™ QuantStudio 5 qPCR thermal cycler. Results confirm that the eluted gDNA is highly pure and free from inhibitors, optimal for qPCR.

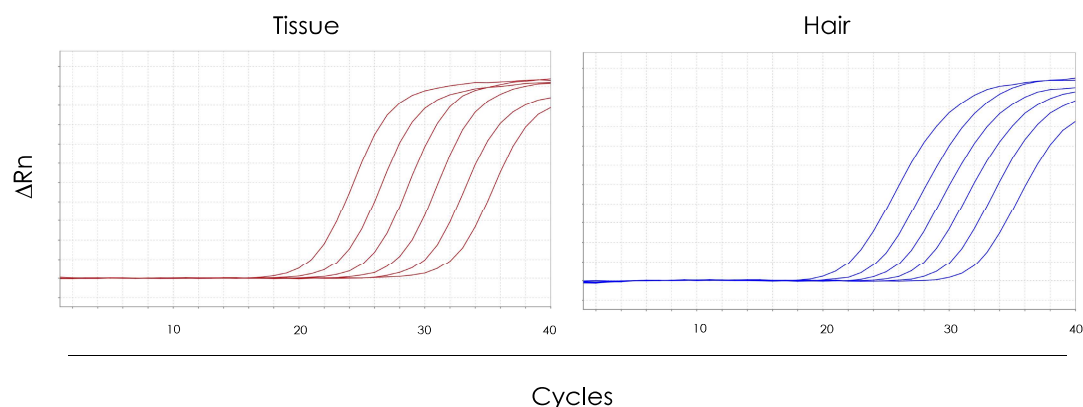


Figure 3. qPCR data using species-specific designed assays.

11. SAMPLE STORAGE AND HANDLING GUIDELINES

Sample Storage

To ensure a high quality of isolated gDNA, fast processing of samples is highly recommended. Overall quality and prior storage conditions of the starting material have a high impact on the ability to obtain suitable gDNA. Exposure to e.g. high temperatures for a considerable period of time may lead to diminished yield and/or (partly) unusable isolated gDNA regarding downstream applications.

Tissue samples

- Fresh tissue samples should be processed immediately or stored for short-term at +4 to +8°C, alternatively frozen at -20°C for long-term (avoid defrosting).
- Frozen tissue samples can be stored at -80°C as whole pieces, already cut into small pieces or tissue powder obtained by pulverization using liquid nitrogen.
- Stabilizing agents (e.g. PhoenixProtect DNA/RNA Conservation Solution, Procomcure Biotech #PCCSKU16009) can be used for transportation (either at room temperature or on ice) or allowing safe mid-term storage at +4 or -20°C.

Hair samples

- Storage and preservation of hair roots and follicles is highly recommended under dry conditions.
- Short-term storage: refrigerated at +4 to +8°C or frozen at -20° C or lower (avoid defrosting)
- Long-term storage: -80°C

Sample Handling

To ensure optimal yield of isolated gDNA, use the recommended amount of starting material (Table 3).

Tissue samples

In order to prevent partial gDNA degradation, homogenization or breakup of tissue samples is of utmost importance. If homogenization is no option, tissue can be pulverized using liquid nitrogen or cut into the smallest possible pieces. Tissue disruption allows for a rapid inactivation of nucleases by Proteinase K. Always use pre-cooled reaction tubes and work on ice until sample lysis.

Fresh and frozen tissue

Keep fresh samples on ice and frozen samples frozen (e.g. on dry ice). Cut tissue into small pieces, weigh in appropriate amount and transfer the pieces to a pre-cooled reaction tube. To avoid thawing of frozen tissue during cutting, process samples ideally on a frozen cooling block.

Frozen tissue powder

Keep tissue powder at -80°C or on dry ice to ensure that the sample does not thaw. Weigh in the appropriate amount of tissue powder in a pre-cooled reaction tube. Avoid thawing of sample by quick processing. Note: It is critical to start lysis immediately after adding Lysis Buffer and Proteinase K to the sample followed by appropriate mixing.

Stabilized tissue

If frozen, thaw stabilized tissue samples before processing. Remove the stabilizing agent from the external part of the tissue (e.g. with an absorbent paper). Perform homogenization of tissue (alternatively cut the stabilized tissue into small pieces). Weigh in the recommended sample amount and transfer to a pre-cooled reaction tube.

Hair samples

In order to obtain optimal results, cut hairs including bulbs into smallest possible pieces and transfer to a reaction tube.

12. PRE-APPLICATION PREPARATION

Make sure to get familiar with the complete extraction process before getting started.

GENERAL IMPORTANT NOTES BEFORE GETTING STARTED

- Prepare Wash Buffer I (only for Universal 96) by adding Isopropanol $\geq 99.5\%$ (not supplied) as indicated on the bottle label and store at room temperature.
- Prepare Wash Buffer II (only for Universal 96) by adding Ethanol, absolute (not supplied) as indicated on the bottle label and store at room temperature.
- When using an alternative to a thermal mixer (e.g. heating block, incubator or water bath), vortex sample at a repetitive frequency for approx. 5 min (depending on the overall incubation time) during initial lysis step.
- Lysis Buffer, Binding Buffer and Wash Buffer I may form precipitates when stored under cool conditions. Check individually bottled buffers for precipitation before use. Re-dissolve at $+37^{\circ}\text{C}$ and gently mix, if necessary.
- For the optional treatment with RNase A (not supplied), prepare an appropriate working solution - recommended concentration: 20mg/ml RNase A.

13. ELUTION AND STORAGE

Buffer Composition

The Elution Buffer is 10 mM Tris-HCl, pH 8.0. Alternatively, nuclease-free water or other low ionic strength buffers can be used.

Temperature

Elution is strongly recommended with the Elution Buffer pre-heated to $+60^{\circ}\text{C}$ to improve elution efficiency.

Volume

The recommended elution volume ranges between 50-100 μl . A higher elution volume results in higher yield, but decreased gDNA concentration. A lower volume allows for higher concentration.

Storage

The eluted gDNA can be safely stored at $+4$ to $+8^{\circ}\text{C}$ for weeks to months. For long term storage, store the eluted gDNA at -80°C . Repeated freeze-thaw cycles should be avoided to maintain eluate integrity.

14. EXTRACTION PROTOCOLS

14.1. AUTOMATED: PHOENIX-PURE 96/AUTO-PURE 96

Compatible Kits: SphaeraMag® Genomic DNA Tissue and Hair Purification Kit - Universal 96
 SphaeraMag® Genomic DNA Tissue and Hair Purification Kit - Pre-filled 96

Important:

- Vortex Magnetic Beads thoroughly for 30 sec to fully resuspend the beads before each use.
- Tip combs and pre-filled plates are single use only. Do not re-use them.
- Make sure that solutions are at the bottom of each well and not on the seal. If necessary, centrifuge the deep-well plates at 2,000 xg for 3 min to remove droplets from the seal.
- The total volume in each well should not exceed 900 µl to avoid spilling and cross-contamination.
- Set a thermal mixer or an alternative heating device to +56°C.

1. Make sure to have V-bottom 96 deep-well plates containing buffers (Table 4) ready for use.

Step Name	Plate Position	Reagents	Volume per well
Loading	1	Tip comb	-
Binding	2	Binding Buffer	200 µl
-	3	-	-
Wash 1	4	Wash Buffer I	600 µl
Wash 2	5	Wash Buffer II	600 µl
Wash 3	6	Wash Buffer II	600 µl
-	7	-	-
Elution	8	Elution Buffer	100 µl

Table 4. Setup for V-bottom 96 deep-well plates compatible with Phoenix-Pure 96/Auto-Pure 96 device.

- For each processed sample, transfer required amount of sample material to a 1.5 ml or 2 ml microcentrifuge tube:
 - Tissue: Up to 100 mg (prior homogenization or cutting into smaller pieces strongly recommended)
 - Hair: Up to 20 hairs incl. bulbs (cut into smallest possible pieces)
- Add 200 µl Lysis Buffer and 20 µl Proteinase K. Mix by vigorous vortexing for 10 sec.
- Incubate in a thermal mixer at +56°C with agitation at full speed (~1,400 rpm): 30 min for tissue samples, 60 min for hair samples.
- (Optional) Add 3 µl RNase A and mix by pulse vortexing. Incubate in a thermal mixer at +56°C with agitation at full speed (~1,400 rpm) for 5 min.
- Remove sealing foil from pre-filled plates.
- Transfer lysate(s) and Magnetic Beads (**orange**) to appropriate well(s) (Table 5) on Position-2 Plate (Binding Buffer).

Step Name	Plate Position	Samples/Reagents	Volume per well
Loading	1	Tip comb	-
Binding	2	Binding Buffer Lysate Magnetic Beads	200 µl ~200 µl 10 µl
-	3	-	-
Wash 1	4	Wash Buffer I	600 µl
Wash 2	5	Wash Buffer II	600 µl
Wash 3	6	Wash Buffer II	600 µl
-	7	-	-
Elution	8	Elution Buffer	100 µl

Table 5. Pipetting scheme for lysate and Magnetic Bead transfer into V-bottom 96 deep-well plates.

8. Load V-bottom 96 deep-well plates onto instrument's plate positions according to plate layout (Table 6). Note: Make sure that all plates are placed in the correct orientation.
9. Set program according to Table 6.

step	step name	plate	mix time (min)	mix amp (%)	wait time (min)	volume (µl)	mix speed (1-10)	temp (°C)	segment (1-5)	cycle time (1-10)	magnet speed (1-10)	1 st segment time (sec)	2 nd segment time (sec)
1	Load	1	0	1	0	5	1	0	1	1	1	1	1
2	Bind	2	5	80	0	500	3	37	2	1	4	20	20
3	Wash 1	4	1	80	0	600	5	0	2	1	3	20	20
4	Wash 2	5	1	80	0	600	5	0	2	1	3	20	20
5	Wash 3	6	2	80	2.5	600	5	0	2	1	3	20	20
6	Elution	8	1	80	0	100	3	60	2	2	3	30	30
7	Unload	1	0	1	0	5	1	0	1	1	1	1	1

Table 6. Program setting for "Genomic DNA Tissue and Hair Purification" on Phoenix-Pure 96/Auto-Pure 96 device.

10. Run the program.
11. After the run is finished, remove V-bottom 96 deep-well plates and tip comb. Transfer eluates from Position-8 plate (Elution Buffer) into new tubes, if required. Process eluates directly or store at +4 to +8°C for short-term or at -80°C for long-term storage.

14.2. AUTOMATED: PHOENIX-PURE 32/AUTO-PURE 32

Compatible Kits: SphaeraMag® Genomic DNA Tissue and Hair Purification Kit - Universal 96
SphaeraMag® Genomic DNA Tissue and Hair Purification Kit - Pre-filled 32

Important:

- Vortex Magnetic Beads thoroughly for 30 sec to fully resuspend the beads before each use.
- Tip combs and pre-filled plates are single use only. Do not re-use them.
- Make sure that solutions are at the bottom of each well and not on the seal. If necessary, centrifuge the deep-well plate(s) at 2,000 xg for 3 minutes to remove droplets from the seal.
- The total volume in each well should not exceed 900 µl to avoid spilling and cross-contamination.
- Set a thermal mixer or alternative heating device to +56°C.

1. Make sure to have U-bottom 96 deep-well plate(s) containing buffers (Table 7) ready for use.

Well	Reagents	Volume per well
Column 1/7	Binding Buffer	200 µl
Column 2/8	-	-
Column 3/9	Wash Buffer I	600 µl
Column 4/10	Wash Buffer II	600 µl
Column 5/11	Wash Buffer II	600 µl
Column 6/12	Elution Buffer	100 µl

Table 7. Setup for an U-bottom 96 deep-well plate compatible with Phoenix-Pure 32/Auto-Pure 32 device.

2. For each processed sample, transfer required amount of sample material to a 1.5 ml or 2 ml microcentrifuge tube:
 - a. Tissue: Up to 100 mg (prior homogenization or cutting into smaller pieces strongly recommended)
 - b. Hair: Up to 20 hairs incl. bulbs (cut into smallest possible pieces)
3. Add 200 µl Lysis Buffer and 20 µl Proteinase K. Mix by vigorous vortexing for 10 sec.
4. Incubate in a thermal mixer at +56°C with agitation at full speed (~1,400 rpm): 30 min for tissue samples, 60 min for hair samples.
5. (Optional) Add 3 µl RNase A and mix by pulse vortexing. Incubate in a thermal mixer at +56°C with agitation at full speed (~1,400 rpm) for 5 min.
6. Remove sealing foil from pre-filled plates.
7. Transfer lysate(s) and Magnetic Beads (**orange**) to appropriate well(s) in columns 1/7 (Table 8).

Well	Samples/Reagents	Volume per well
Column 1/7	Binding Buffer Lysate Magnetic Beads	200 µl ~200 µl 10 µl
Column 2/8	-	-
Column 3/9	Wash Buffer I	600 µl
Column 4/10	Wash Buffer II	600 µl
Column 5/11	Wash Buffer II	600 µl
Column 6/12	Elution Buffer	100 µl

Table 8. Pipetting scheme for lysate and Magnetic Bead transfer into an U-bottom 96 deep-well plate.

8. Load an U-bottom 96 deep-well plate(s) onto the instrument.
Note: Make sure that plates are placed in the correct orientation.
9. Place new clean tip comb(s) in the instrument.
10. Set program according to Table 9.

step	step name	well	mix time (min)	magnet (sec)	wait time (min)	volume (µl)	mix speed (1-10)	temp (°C)	mix pos (%)	mix amp (%)	magnet pos (%)	magnet speed (1-10)
1	Bind	1	5	68	0	500	4	37	0	80	0	4
2	Wash 1	3	1	68	0	600	5	0	0	80	0	3
3	Wash 2	4	1	68	0	600	5	0	0	80	0	3
4	Wash 3	5	2	68	2.5	600	5	0	0	80	0	3
5	Elution	6	1	180	0	100	6	60	0	80	0	3
6	Waste	1	0.1	0	0	500	1	0	0	80	0	3

Table 9. Program setting for "Genomic DNA Tissue and Hair Purification" on Phoenix-Pure 32/Auto-Pure 32 device.

11. Run the program.
12. After the run is finished, remove the U-bottom 96 deep-well plate(s) and tip comb(s). Transfer the eluate(s) from the column(s) 6/12 to new tubes, if required. Process eluates directly or store at +4 to +8°C for short-term or at -80°C for long-term storage.

14.3. AUTOMATED PURIFICATION: AUTO-PURE MINI

Compatible Kit: SphaeraMag® Genomic DNA Tissue and Hair Purification Kit - Universal 96
SphaeraMag® Genomic DNA Tissue and Hair Purification Kit - Pre-filled Mini

Important:

- Vortex Magnetic Beads thoroughly for 30 sec to fully resuspend the beads before each use.
- Tip combs and pre-filled cartridges are single use only. Do not re-use them.
- Make sure that solutions are at the bottom of each well and not on the seal. When using a V-bottom 96 deep-well plate, centrifuge the deep-well plates at 2,000 xg for 3 minutes to remove droplets from the seal, if necessary.
- The total volume in each well should not exceed 900 µl to avoid spilling and cross-contamination.
- Set a thermal mixer or alternative heating device to +56°C.

Samples can be processed **(a)** in cartridges (1 cartridge per sample) or **(b)** in a V-bottom 96 deep-well plate (16 samples per plate). Make sure to have buffers dispensed according to Table 10 or Table 11.

(a) Cartridge format:

Well	Reagents	Volume per well
Position 1	Binding Buffer	200 µl
Position 2	-	-
Position 3	Wash Buffer I	600 µl
Position 4	Wash Buffer II	600 µl
Position 5	Wash Buffer II	600 µl
Position 6	Elution Buffer	100 µl

Table 10. Setup for cartridge(s) compatible with Auto-Pure Mini.

(b) V-bottom 96 deep-well format:

Well	Reagents	Volume per well
Column 1/7	Binding Buffer	200 µl
Column 2/8	-	-
Column 3/9	Wash Buffer I	600 µl
Column 4/10	Wash Buffer II	600 µl
Column 5/11	Wash Buffer II	600 µl
Column 6/12	Elution Buffer	100 µl

Table 11. Setup for a V-bottom 96 deep-well plate compatible with Auto-Pure Mini.

1. For each processed sample, transfer required amount of sample material to a 1.5 ml or 2 ml microcentrifuge tube:
 - a. Tissue: Up to 100 mg (prior homogenization or cutting into smaller pieces strongly recommended)
 - b. Hair: Up to 20 hairs incl. bulbs (cut into smallest possible pieces)
2. Add 200 µl Lysis Buffer and 20 µl Proteinase K. Mix by vigorous vortexing for 10 sec.
3. Incubate in a thermal mixer at +56°C with agitation at full speed (~1,400 rpm): 30 min for tissue samples, 60 min for hair samples.
4. (Optional) Add 3 µl RNase A and mix by pulse vortexing. Incubate in a thermal mixer at +56°C with agitation at full speed (~1,400 rpm) for 5 min.

- Remove sealing foil from cartridge(s) or from the V-bottom 96 deep-well plate.
- Transfer lysate(s) and Magnetic Beads (**orange**) to appropriate well(s) (Table 12 or Table 13).

(a) Cartridge format:

Well	Samples/Reagents	Volume per well
Position 1	Binding Buffer Lysate Magnetic Beads	200 μ l ~200 μl 10 μl
Position 2	-	-
Position 3	Wash Buffer I	600 μ l
Position 4	Wash Buffer II	600 μ l
Position 5	Wash Buffer II	600 μ l
Position 6	Elution Buffer	100 μ l

Table 12. Pipetting scheme for lysate and Magnetic Bead transfer into cartridge(s).

(b) V-bottom 96 deep-well format:

Well	Samples/Reagents	Volume per well
Column 1/7	Binding Buffer Lysate Magnetic Beads	200 μ l ~200 μl 10 μl
Column 2/8	-	-
Column 3/9	Wash Buffer I	600 μ l
Column 4/10	Wash Buffer II	600 μ l
Column 5/11	Wash Buffer II	600 μ l
Column 6/12	Elution Buffer	100 μ l

Table 13. Pipetting scheme for lysate and Magnetic Bead transfer into the V-bottom 96 deep-well plate.

- Load cartridge(s) or the V-bottom 96 deep-well plate onto instrument. Note: Make sure that cartridge(s) or the V-bottom 96 deep-well plate are placed in the correct orientation.
- Place new clean tip comb(s) in the instrument.
- Set program according to Table 14.

step	step name	hole site	volume (μ l)	mix time (min)	mix speed	dry time (min)	temperature ($^{\circ}$ C)	segments	every time (sec)	magnetization time (sec)	cycle	magnet speed (mm/sec)	mix scope (%)	mix pos.	magnet pos.
1	Bind	1	500	5	7	0	37	2	30	0	1	3	80	0	0
2	Wash 1	3	600	1	5	0	0	2	30	0	1	3	80	0	0
3	Wash 2	4	600	1	5	0	0	2	30	0	1	3	80	0	0
4	Wash 3	5	600	2	5	2.5	0	2	30	0	1	3	80	0	0
5	Elution	6	100	1	4	0	60	2	45	0	2	3	80	0	0
6	Waste	1	500	0.1	5	0	0	1	0	0	1	2.5	80	0	0

Table 14. Program setting for "Genomic DNA Tissue and Hair Purification" on Auto-Pure Mini device.

- Run the program.
- After the run is finished, remove cartridge(s) or V-bottom 96 deep-well plate and tip comb(s). Transfer the eluate from Position 6 or column(s) 6/12 to new tubes, if required. Process eluates directly or store at +4 to +8 $^{\circ}$ C for short-term or at -80 $^{\circ}$ C for long-term storage.

14.4. MANUAL EXTRACTION

Preparation:

- Pre-heat appropriate volume of Elution Buffer to +60°C.
1. Transfer required amount of sample to a 1.5 ml or 2 ml microcentrifuge tube:
 - a. Tissue: Up to 100 mg (prior homogenization or cutting into smaller pieces strongly recommended)
 - b. Hair: Up to 20 hairs incl. bulbs (cut into smallest possible pieces)
 2. Add 200 µl Lysis Buffer and 20 µl Proteinase K. Mix by vigorous vortexing for 10 sec.
 3. Incubate in a thermal mixer at +56°C with agitation at full speed (~1,400 rpm): 30 min for tissue samples, 60 min for hair samples.
 4. (Optional) Add 3 µl RNase A and mix by pulse vortexing. Incubate in a thermal mixer at +56°C with agitation at full speed (~1,400 rpm) for 5 min.
 5. Vortex Magnetic Beads thoroughly. Add 200 µl Binding Buffer and 10 µl Magnetic Beads to the tube. Mix by pipetting up and down at least 5 times.
 6. Incubate in a thermal mixer at +37°C with agitation at full speed (~1,400 rpm) for 5 min. Alternative: Incubate in heating device at +37°C for 5 min. Invert the tube 2 times during incubation.
 7. Place the tube on a magnetic stand. Allow Magnetic Beads to settle until the solution is completely clear.
 8. Remove and discard the supernatant. Avoid disturbing the Magnetic Bead pellet.
 9. Add 600 µl Wash Buffer I.
 10. Remove the tube from the magnetic stand. Mix well by pipetting several times up and down.
 11. Place the tube on the magnetic stand. Allow Magnetic Beads to settle until the solution is completely clear.
 12. Remove and discard the supernatant. Avoid disturbing the Magnetic Bead pellet.
 13. Add 600 µl Wash Buffer II.
 14. Remove the tube from the magnetic stand. Mix well by pipetting several times up and down.
 15. Place the tube on the magnetic stand. Allow Magnetic Beads to settle until the solution is completely clear.
 16. Remove and discard the supernatant. Avoid disturbing the Magnetic Bead pellet.
 17. Repeat steps 13-16 for an additional washing step with Wash Buffer II.
 18. Remove any residual liquid. Leave the tube open for 5 min to allow Magnetic Beads air dry.
 19. Add 100 µl pre-heated Elution Buffer to the tube.
 20. Remove the tube from the magnetic stand. Mix well by pipetting up and down ~10 times.
 21. Incubate at room temperature for 1 min.
 22. Place the tube on the magnetic stand. Allow Magnetic Beads to settle until the solution is completely clear.
 23. Transfer the eluate to a clean tube. Process directly, store at +4 to +8°C for short-term or at -80°C for long-term storage.

15. TROUBLESHOOTING GUIDE

Please use this guide to troubleshoot possible problems that may arise. For further assistance, please contact the technical support staff at sales@procomcure.com.

Problem	Possible Cause	Solution
Low DNA Yield	Incomplete resuspension of SphaeraMag® Magnetic Beads	Thoroughly resuspend SphaeraMag® Magnetic Beads before use.
	DNA degradation during sample handling or storage	Process sample immediately after collection or removal from adequate storage.
	Incorrect preparation of Wash Buffers	Prepare Wash Buffers by adding the correct amount of Ethanol or Isopropanol according to instructions indicated on the bottle labels.
	Too much protein present in starting material (excess protein may compete with gDNA for binding to Magnetic Beads)	Use less sample.
	Insufficient sample material	Increase lysis and binding time if the sample is diluted.
	Insufficient digestion	Tissue pieces are too large. Cut tissue into the smallest possible pieces.
Problems with downstream applications	Poor DNA quality	Do not freeze-thaw the isolated gDNA more than once or store at room temperature.
	Insufficient gDNA was used	Quantify the isolated gDNA accurately and use sufficient gDNA.
	Ethanol carry-over	Dry the SphaeraMag® Magnetic Beads completely before adding Elution Buffer.
Carry-over of Magnetic Beads	Incomplete magnetization	Place the eluted samples on a magnetic stand device for additional 5 minutes or centrifuge at >4,000 xg for 5 minutes. When using automated isolation, check the instrument settings and increase bead binding time, if necessary.

Table 15. Troubleshooting guide.

16. CONSUMABLES AND RELATED PRODUCTS

Product	Content	Cat.No.
SphaeraMag® Genomic DNA Tissue and Hair Purification Kit - Universal 96	96 preps	PCCSKU16088
SphaeraMag® Genomic DNA Tissue and Hair Purification Kit - Pre-filled 96	96 preps for Phoenix-Pure 96 & Auto-Pure 96	PCCSKU16089
SphaeraMag® Genomic DNA Tissue and Hair Purification Kit - Pre-filled 32	80 preps for Phoenix-Pure 32 & Auto-Pure 32	PCCSKU16090
SphaeraMag® Genomic DNA Tissue and Hair Purification Kit - Pre-filled Mini	48 preps for Auto-Pure Mini	PCCSKU16091
Tip Combs for Auto-Pure 96 & Phoenix-Pure 96	2 pcs	PCCSKU30014SA
Tip Combs for Auto-Pure 32, Phoenix-Pure 32 & Auto-Pure Mini	20 pcs	PCCSKU16013
U-Bottom Deep-well Plates for Auto-Pure 32 & Phoenix-Pure 32	20 pcs	PCCSKU16014
V-Bottom Deep-well Plates for Auto-Pure 96, Phoenix-Pure 96 & Auto-Pure Mini	50 pcs	PCCSKU30002

Table 16. Consumables and related products.

Ordering information

For ordering SphaeraMag® Genomic DNA Tissue and Hair Purification Kit and other products, visit us at

www.procomcure.com

or order via E-mail:

sales@procomcure.com



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